SPERM AGGLUTINATION AND FERTILIZATION.

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In a recent paper on "Cluster Formation of Spermatozoa Caused by Specific Substances From Eggs" Loeb ('14) has presented a criticism of my theory of fertilization (Lillie '13*b* and '14), based on observation of the California sea-urchin *Strongylocentrotus purpuratus*. My own observations were made on *Arbacia punctulata* of Massachusetts, and it would appear that part at least of Loeb's criticism was due to certain differences in the two forms, for he has now stated (Loeb, 1914*b*, p. 318, footnote) that the "cluster formation" of the spermatozoa may find its explanation "on the assumption of an agglutination at least in the case of *Arbacia*," as I maintained; it is therefore not a "tropistic reaction" as he thought probable from his observations in California. This was one of the chief differences of opinion. A second one was in regard to the source and significance of the substance in the fluid of egg suspensions that caused such agglutination; Loeb maintained that it was merely the dissolved chorion (*i. e.*, jelly layer) of the egg, and that after this was removed the eggs no longer produced the agglutinating substance, and yet were capable of fertilization; whereas my contention was that the agglutinating substance was a secretion of the egg soaked up by the jelly, as by a sponge; that the eggs produced it for a certain length of time after the removal of the jelly, and lost their power of fertilization after they ceased to produce it.

These criticisms cut at the foundation of my theory. Inasmuch as the correction of the tropistic interpretation of agglutination is given only in a footnote to another paper, and no correction of the source of the agglutinating substance has yet appeared, it is incumbent on me to consider the criticisms carefully; at the same time I wish to take the opportunity to explain certain points that appear to be open to misinterpretation, and to record some new observations.

1. "Cluster-formation" versus Agglutination.

The phenomena exhibited by sperm suspensions of *Arbacia* with which we have to deal are of four distinct types, which it is essential to distinguish sharply: (1) activation; (2) aggregation; (3) agglutination, (4) mass-coagulation.[1] (1) That the activity of spermatozoa is affected by substances in the sea-water requires no argument. The subject is discussed in study V (Lill'e, 1913*a*, pp. 519–532). (2) Aggregation of spermatozoa may be brought about by tropistic reactions. In my paper on the "Behavior of Spermatozoa," I devoted a great deal of attention to such aggregation phenomena and the distinction from phenomena of agglutination (1913, pp. 532–548 and pp. 551–552). Among other things I pointed out that aggregation as a tropistic phenomenon implies a gradient,[2] and that the spermatozoa never adhere, however crowded they may be; there is no observable physical change of the spermatozoa and the slightest agitation suffices to disperse them again. Such tropistic phenomena may be exhibited in response to CO_2 and other acids (*Nereis*), or certain constituents of egg secretions, to mention only chemotaxis.

(3) Agglutination of spermatozoa on the other hand requires no gradient, and the spermatozoa adhere physically to such an extent that the agglutinated masses may be preserved intact in killing fluids; its degree is a function of the concentration of the agglutinating medium, and is also different in different species. Agglutination is non-toxic, not limiting the life of the spermatozoa; it is reversible, its duration depending on the concentration of the agglutinating medium; it cannot be repeated if the reaction is complete, at least within the time limits of my experiments, even though the spermatozoa remain motile;[3] finally motility of the spermatozoa is a prerequisite to a decided reac-

[1] The reaction here referred to is a lethal phenomenon. It possibly involves cytolysis with subsequent adhesion of the spermatozoa.

[2] It is important to notice that the spermatozoa of suspensions may produce gradients through their own activities. Thus I pointed out that autogenous aggregation reactions in sperm suspensions of *Nereis* arise from the positive chemotaxis of the spermatozoa to their excreted CO_2, giving rise to very striking phenomena (Lillie, 1913*a*, pp. 519–521 and pp. 538–540). It is conceivable that such a tropistic phenomena is involved as a part factor in the agglutination phenomena under discussion.

[3] Glaser (1914) also comes to this conclusion.

tion; evidently because the physical change on which the reaction depends is not sufficient to cause adhesion except when the spermatozoa positively collide.[1] These six criteria definitely define the phenomenon.

Agglutination is positively distinct from aggregation. It is an entirely different biological phenomenon. The two may, however, be exhibited simultaneously, as when a drop of egg secretion of *Arbacia* is injected into a sperm suspension of the species. In such a case the spermatozoa exhibit positive chemotaxis to one constituent of the egg secretion, and are agglutinated by another (the fertilizin). The separateness of these two substances was maintained in my first publication on the subject and demonstrated by repeated experiments (see Lillie, 1913*a*, p. 549, and 1914, pp. 545–546).

(4) The phenomenon of mass-coagulation is, on the other hand, a lethal irreversible phenomenon. It may be exhibited in response to various agents, such as KOH, NaOH, salts of lanthanum and cerium,[2] etc., and in some cases the secretions of the eggs of other species or their blood. Hitherto I have not adequately defined this phenomenon as distinct from the agglutination phenomena, though in my last study (1914), I noted the distinction (p. 541). The phenomenon is essentially lethal, but not all destructive agents exhibit it; thus acids, so far as I have observed, destroy the spermatozoa without causing mass coagulation. The phenomenon is irreversible, and this suffices to distinguish it from true agglutination, even if no other criterion were available. However, it exhibits quite a different aspect from agglutination; in the latter the sperm masses tend to take on a spherical form; if originally elongated they contract into balls or break up into smaller masses which become spherical, thus offering considerable resemblance to a phenomenon of surface tension, as Loeb notes. The peripheral spermatozoa are in violent movement until the time of reversal. In the mass-coagulation reaction, on the other hand, there is no such surface tension effect, strands anastomose

[1] Loeb argues that the necessity of movement on the part of the spermatozoa for the appearance of this phenomenon removes it from the category of true agglutination; but this seems to me to be a purely arbitrary criterion.

[2] My attention was called to the action of the salts of these metals by a letter from James Gray of Cambridge University.

with other strands and form a net-work and the movements of the spermatozoa soon cease.

The substances of egg secretions, which I have hitherto called hetero-agglutinins, belong to this category, in some cases at any rate. Though I will not assert that there is no such substance as a hetero-agglutinin in the real sense of agglutination, yet the substance in *Arbacia* blood, or egg secretions, the effects of which on *Nereis* sperm I have previously studied, should be regarded as a toxic rather than an agglutinating substance, having the mass coagulant action. As I stated in my last paper, p. 541, it produces a permanent coagulum in *Nereis* sperm suspensions; "in this respect the action differs from the iso-agglutination, which is without toxic effects."

We must keep firmly in mind the distinctions between aggregation (tropisms) agglutination, and mass-coagulation. Agglutination, with which we are particularly concerned, is distinguished from aggregation by the facts that it occurs in the absence of a gradient, it involves physical adhesion, and cannot be repeated if the reaction is once complete; it also is characterized by a high degree of specificity.[1] From mass coagulation it is distinguished by the facts (1) that it is non-toxic, (2) reversible, (3) dependent on motility of the spermatozoa. Agglutination occurs so far as I have observed with certainty only in response to egg-secretions of the same species.

For description of the phenomena of agglutination of sperm by egg-extractives of the same species, I must refer to my previous paper (Lillie, 1913*a*); the phenomenon in *Arbacia* is a true agglutination in the sense defined, not a tropistic reaction, nor yet a mass coagulation. Loeb has admitted this for *Arbacia*, and I would therefore venture to suggest the probability that the phenomenon which Loeb has described in *Strongylocentrotus* and termed "cluster formation," which he interprets with some reserve as a possible tropistic reaction, is also true agglutination, which differs only quantitatively from *Arbacia* and *Nereis*. The

[1] Loeb admits that the "cluster formation" exhibits a high degree of specificity. It is therefore inconsistent to interpret the reaction, as he also does, as a "possible tropistic phenomenon" because such phenomena so far as we know do not exhibit specificities of this kind. Agglutination phenomena, on the other hand, as is well known, commonly exhibit equal specificity of a similar kind.

conditions under which it occurs, in response to egg secretions of the same species, its character, reversibility, and the specificity of the reaction are identical with *Arbacia*. It is apparently, however, less pronounced, and therefore not so readily recognizable of itself as an agglutination phenomenon. Even the "apparent surface tension phenomena" which Loeb describes for the clusters—"Short streaks or cylinders contract into spherical masses, the above described clusters; and long cylinders break up into a series of small clusters"—are the same as I previously described for *Arbacia* (1913*a*, pp. 550–551).

Loeb's interpretation of the "cluster-formation" as a possible tropistic reaction confuses the two sets of phenomena—viz., aggregation (a true tropistic phenomenon) and agglutination—which sperm suspensions may exhibit to the egg-sea-water of its own species. But the aggregation (tropism) can take place only when there is a gradient from the secretion to the spermatozoa. This is realized under the conditions of my experiment of injecting a drop of egg-sea-water into a fresh sperm suspension beneath a raised cover slip; in such a case the two phenomena take place simultaneously viz.; aggregation in the form of a ring around or in the introduced drop (depending on concentration), and agglutination. These two phenomena are produced by two constituents of the egg-sea-water, as I have already maintained.

For the study of the aggregation phenomena therefore it is desirable to employ an agent which has no agglutinative action. This I did in an extensive series of experiments by the method just referred to (1913, p. 533 ff.). To illustrate:—a drop of a $^1/_{100}$ dilution of a saturated solution of CO_2 in sea-water injected into a sperm suspension of *Nereis* in sea-water mounted beneath a raised cover-slip is marked within a few seconds by the formation of a ring of active spermatozoa within the margin of the introduced drop, and separated from the general sperm suspension by a clear zone nearly free of spermatozoa 1.5 to 2 mm. in diameter. I interpreted the ring formation as a positive reaction to the attractive substance (CO_2 and acids generally); the spermatozoa follow the gradient from the suspension into the drop containing CO_2 a certain distance, *i. e.*, up to a certain concentration, and

are there arrested. The proof of this interpretation is found in the fact that, if increasing concentrations of CO_2 are used, the ring forms outside the drop and becomes progressively wider, *i. e.*, the migration ceases at a distance from the center which increases with CO_2 or acid concentration (see 1913*a*, pp. 536–538). Loeb suggests that the ring formation with a clear external zone around it is "an indication that the spermatozoa are negatively chemotropic to the strong egg-sea-water, and possibly positively chemotropic to the more diluted egg-sea-water, or to the dense collection of spermatozoa in the ring." The latter suggestion is of course untenable as a primary cause, for the ring-formation is precisely the phenomenon to be explained. It is also unnecessary to assume any negative tropism; the ring formation is due to a limitation of the positive movement by concentration. This is fully discussed in the paper referred to above, but Loeb does not allude to the discussion.

2. The Source of the Agglutinating Substance.

Professor Loeb has also taken issue with me on the question of the origin of the agglutinating substance. He regards his experiments as proving that the substance which causes the "cluster formation" is not formed in the egg but in the chorion; *i. e.*, in the layer of jelly which surrounds the egg. On the other hand I regarded it (and still hold to the opinion) as a secretion of the egg; with which the jelly of course becomes saturated.[1] Loeb's observations again were on *Strongylocentrotus* and mine on *Arbacia*. The issue is a real one even though the chorion is itself a secretion of the egg in earlier stages.

Loeb's conclusion was based on his observation that if the chorion be dissolved off in dilute hydrochloric acid in sea-water, the naked eggs transferred to sea-water produce no detectable amounts of the agglutinating substance any more, whereas the acid sea-water contains it in large quantities. My conclusions were based on the observation that when eggs of *Arbacia* are deprived of jelly (chorion) by shaking, or a prolonged series of

[1] Glaser (1914) also agrees substantially with me: "the agglutinating substance is located in greatest abundance in the jelly and the eggs also contain this material," p. 371.

washings, they still continue to produce the agglutinating substance in sea-water, though in much diminished quantity; in my full paper, which Loeb had not the opportunity of consulting, I gave series of measurements on this point (1914, pp. 532–538); I also pointed out that in immature ovaries containing many primary ovocytes, but some mature eggs, the quantity of agglutinating substance produced was relatively very small (1914, p. 530), and I therefore suggested that the substance was secreted by the eggs at the time of maturation and was soaked up by the jelly as by a sponge. The eggs, however, continue to produce it after maturation, as I shall show. The immature eggs have as thick a chorion as the mature eggs; therefore the agglutinating substance cannot be merely dissolved chorion. I recognized the possibility of the view expressed by Loeb, investigated it as fully as possible at the time, and rejected it.

Since Loeb's paper has appeared, I have repeated his experiments and found my former observations and conclusions confirmed in all respects:

Experiments.—The optimum concentration of HCl for removal of jelly without injury to eggs was found to be 50 c.c. sea-water + 1.4 c.c. N/10 HCl. 1.2 c.c. N/10 HCl in 50 c.c. sea-water did not fully remove the jelly, and 1.6 c.c. caused too much injury to the eggs evidenced by heavy agglutination and later cytolysis. In an experiment of July 17, 1914, the three above concentrations were used. The complete removal of the chorion in the intermediate concentration was demonstrated by observation of the eggs in a thick suspension of India ink in sea-water; even the minutest traces of adherent jelly can readily be detected by this method, but it was all gone. The eggs were then washed as follows: 10.11 A.M. 42/6 c.c.; 10.40 51/5 c.c.; 10.58 50/4 c.c. The supernatant fluid was then tested and found to be free from sperm agglutinating substance; thus furnishing proof that all originally contained in the jelly had been washed out. At 11.20 the supernatant fluid was poured off leaving only 5 c.c. in the tube. The eggs were allowed to settle, and at 11.25 the supernatant fluid was tested and gave a 9–10-second agglutination reaction with fresh sperm suspension. Thus these eggs entirely deprived of jelly by HCl are producing agglutinating substance.

At 4.25 P.M. the eggs were washed again 5/0.7 c.c. and the new fluid gave a 14-second reaction. The next morning the same eggs were washed again 5.5/1 c.c. The new fluid gave a 6–7-second reaction.

These results may be expressed in a different way: thus in an experiment of July 20, a series of eight successive washings of eggs deprived of jelly by acid sea-water represented a dilution of the agglutinating substance contained in the acid sea-water remaining with the eggs of 12,700,800 times. But the acid solvent itself was negative at 1/800 dilution: it was of 400 agglutinating power. In other words, after the removal of the jelly the eggs themselves had produced a sufficient quantity of the agglutinating substance to account for the tremendous difference; and they were still producing it.

These eggs without jelly are fertilizable, as Loeb states, but only 37 per cent. segmented in a heavy insemination of the first day in the experiment of July 17, and only a small part of these developed to the ciliated stage, none of which were normal, most being stereoblastulae and incapable of farther development. The result is entirely similar to that described in my last paper (study VI, '14) for the fertilization of eggs deprived of jelly by shaking and subsequent washing.

The same experiment was repeated on July 18, 20 and 21, with identical results: the eggs from which jelly is entirely removed by HCl continue to produce the sperm-agglutinating substance (fertilizin) so long as they live, but their capacity for development after fertilization is much reduced.

In all experiments at least three concentrations of acid were used, and in each experiment it was observed that when the concentration was sufficient to dissolve the jelly there was a good deal of agglutination of the eggs, and in the later washings a great many eggs broke down liberating their pigment. As I have previously shown, broken-down eggs liberate a substance (anti-fertilizin) which neutralizes the sperm agglutinating action of the fertilizin. Therefore, when a sufficient percentage of the eggs are breaking down, the production of sperm-agglutinating substance (fertilizin) by intact eggs may be entirely masked.

I have no intention of disputing Professor Loeb's observations

for *Strongylocentrotus*. But they merely prove either that *Strongylocentrotus* sperm is not so delicate an indicator as *Arbacia* sperm, or that the method employed by Loeb was inadequate to detect small quantities of fertilizin. In *Arbacia* the eggs continue to charge the sea-water with sperm-agglutinating substance after complete removal of the jelly, whether by shaking and repeated washings, or by HCl; and the substance continues to be formed as long as the eggs remain fertilizable and living, no matter how often the eggs are washed. *The eggs of Arbacia secrete the substance as I previously maintained. It is not merely the "dissolved chorion."*

It might possibly be objected to this conclusion that the continued appearance of the agglutinating substance in egg suspensions in sea-water after removal of the chorion indicated merely previous adsorption of the substance of the chorion. But the indefinite continuance of its production is inconsistent with the idea of a mere secondary removal of an adsorbed substance. The idea is also inconsistent with the fact that *Nereis* eggs have no jelly at the time laying, but produce a similar sperm agglutinating substance. In this form the jelly also is secreted by the egg after insemination.

Finally if it can be shown that the jelly of immature eggs is entirely devoid of the sperm agglutinating substance, my position that this substance is a later secretion of the egg is rigorously proved. As noted above I maintained the probability of this view in my previous paper (Study VI). This summer my first experiments were undertaken to investigate this point anew.

Fortunately the season was late, and not a single *Arbacia* was ripe when I began work (June 8). This applied to males as well as females: so it was impossible at first to secure ripe sperm as indicator. I therefore made extracts of immature ovaries to be kept for subsequent testing from three females (1, 2, and 3, June 8). June 11 extracts of ovaries in sea-water were made from females 4, 5, and 6: numbers 4 and 6 contained only ovocytes; No. 5 had a large number of ripe ova in addition. On June 16 extracts 1–6 were tested with *Arbacia* sperm suspension: 1, 2, 3, 4, and 6 were absolutely negative; no agglutination. No. 5 gave a strong agglutination reaction lasting about one minute.

It is highly improbable that the agglutinating substance had been destroyed in five of the six, and retained in the only one (No. 5) of the extracts which was made from ovaries containing some ripe ova. So far as these observations go, the jelly of immature ovocytes is free of agglutinating substance.

Again on June 15 I made extracts from ovaries of three females in two of which ripe ova were practically absent, the third had a few. Tested the same day the two former extracts had no sperm agglutinating properties; the third gave slight agglutination.

The females appeared to mature slightly earlier than the males, so that for these experiments I was forced to use rather thin sperm suspensions (mixed more or less with immature spermatozoa), which were probably not as delicate indicators as one could wish. However the difference between the ovaries containing ripe ova and those without was perfectly distinct. Later when fully ripe males could be had all ovaries contained ripe ova.

The following observation also tends in the same direction: June 27, 1914—Three females were selected, of which number 1 was the ripest attainable, the eggs flowing freely out of detached ovaries, and very few ovocytes occur; numbers 2 and 3 were the least mature attainable; number 2 had very few detachable ova, mostly late ovocytes with a sprinkling of ripe eggs; number 3 had quite a few detachable ova with a large proportion of ripe eggs. The ovaries of all three were cut up equally, and sea-water added to each to make 10 c.c. When the ova and ovaries had settled they stood at 1.5 c.c. in 1, at 1.3 c.c. in 2, and 1.5 c.c. in 3. After five hours, tests of the agglutinating strength of the supernatant fluids were made with clear fresh sperm.

No. 1 gave a 10-second reaction at 1/800 dilution.

No. 2 gave a 6-second reaction at 1/10 dilution.

No. 3 gave a 7-second reaction at 1/40 dilution.

Thus No. 1 is 80 times the strength of 2 and 20 times the strength of 3. In general the fertilizin production is proportional to the ripeness of the ovaries.

There is not the slightest doubt in my mind about the demonstrative character of these observations. The appearance of agglutinating substance in the jelly of *Arbacia* eggs is secondary,

and takes place probably at the time of breaking down of the germinal vesicle.

Loeb's contention that the agglutinating substance is merely dissolved chorion therefore does not hold for *Arbacia*. With this his argument against my fertilizin theory also falls: "Moreover if it should turn out that the substance which is responsible for the cluster formation is identical with the substance which Lillie calls "fertilizin," which is very likely the case, Lillie's theory becomes untenable, since this substance does not, in all probability, originate from the egg, but from the chorion and since there is, as we have seen, no connection between the presence of this substance and the power of the eggs of being fertilized" (pp. 136–137—Loeb, '14).

In this statement Loeb sums up the essentials of his criticism; since I have shown that "cluster formation" is true agglutination (which Loeb now admits), and that the agglutinating substance (my fertilizin) is not dissolved chorion but a true secretion of the eggs which continues to be produced after the chorion is removed, the entire stated criticism becomes ineffective. There *is* a connection between the presence of this substance and the power of the eggs of being fertilized: the substance can first be demonstrated at the time that the power of being fertilized first arises, viz., after breakdown of the germinal vesicle; it can be demonstrated as long as eggs retain the power of being fertilized, whether the chorion be removed or not, and it disappears absolutely after fertilization, as I showed in my previous paper (study VI, p. 553, 1914).

3. Other Criticisms.

Another objection raised by Loeb is that "the supernatant sea-water of the eggs of *Strongylocentrotus franciscanus* will not induce cluster formation of the sperm of *Strongylocentrotus purpuratus:* yet the latter sperm fertilizes the eggs of *franciscanus*," from which he argues that the fertilizin of *Strongylocentrotus franciscanus* can not be necessary for the fertilization of its eggs. An error in logic is involved here; agglutination of sperm is merely an indicator of the presence of a certain substance, which is none the less present in *franciscanus* even if *purpuratus*

sperm does not reveal it; it *may* nevertheless be activated by *purpuratus* sperm and this is the essential point in the theory.[1] Agglutination of sperm is of no significance except as indicator. As I pointed out in my previous paper, binding of the fertilizin by sperm receptors, *i. e.*, the chemical reaction, is a thing entirely distinct from agglutination; if such binding causes a certain kind of physical surface change of the spermatozoa of suspensions of a certain minimum concentration, they agglutinate; otherwise not. Agglutination is a valuable indicator that enables us to make certain analyses, and that is all. The same principle of fertilization may hold in the entire absence of sperm agglutination.

Another objection in which Loeb supports the possibility of superposing fertilization on parthenogenesis will be dealt with in a separate paper. My contention in this case is that the possibility of such superposition always rests upon incompleteness of the parthenogenetic reaction; if the fertilization reaction be complete, whether by parthenogenesis or insemination, it cannot be repeated. Everybody admits that eggs fertilized by sperm cannot be refertilized; it is a logical impossibility that eggs "fertilized" by parthenogenetic reagents should be refertilized. The problem of the apparent contradiction involved in Loeb's and Herbst's contention of superposition works out in the manner indicated. A study of this problem by one of my students will appear soon.

Loeb cites as a farther difficulty of my fertilizin theory, which he says I have not considered, "that in addition to the membrane forming substance still another, namely a correcting agency, is necessary for causation of the development of the egg." Though

[1] Loeb states (1914, p. 135): "If the phenomenon of cluster formation were inseparably associated with the power of the eggs of being fertilized, we should expect that sperm should only be able to fertilize the eggs of a species if the egg-sea-water of the same species caused the cluster formation of the sperm." I have never maintained that agglutination ("cluster formation") is inseparably associated with the power of the eggs of being fertilized, but merely that a certain substance produced by the egg is a necessary factor in fertilization. In some cases this substance (fertilizin) produces agglutination of the sperm of its own species, and this reaction furnishes an indicator of its amount, when present, or of its absence. In other cases such an indicator is lacking: I do not find that supernatant sea-water of the eggs of the starfish (*Asteria forbesii*), for instance, agglutinates its own sperm; but I have evidence, to be published elsewhere, that the mechanism of fertilization may be explained in the same way as in *Arbacia*.

I cannot accept this statement of the problem, I have nevertheless taken into consideration the fundamental fact, to which Loeb alludes, in the full account of my experiments, which appeared after Loeb's paper was in press. The fundamental fact is simply that the fertilization process in some cases can be divided in two sharply marked stages. This is perhaps most simply and convincingly shown by my own experiment (Lillie, 1911) of removing the spermatozoon from the egg of *Nereis* after it had already induced the cortical changes, with the result that the developmental phenomena came to a standstill before the first cleavage. I cannot agree with Loeb that the second stage involves a factor corrective of an excess action of the factor of the first stage. I think it is probable that we have a progressive process readily capable of resolution into two stages.

In my complete paper (Lillie, 1914) I considered the second phase of fertilization with reference to the new theory, and may refer the reader to the discussion there given (study VI, pp. 582–584). Here it is only necessary to point out that the "fertilizin" theory is at least as well adapted to account for the two stages as the "lysin" theory.

4. Conclusion.

I may be allowed to emphasize the essential features of my theory with some added light thrown by the work of this summer. The fundamental conception is that all agencies initiating development of the egg do so by the same means, viz., activation of an ovogenous substance, which I have termed fertilizin. This conception brings fertilization and parthenogenesis under one conception. I further assumed that such activation in the case of fertilization was caused by union of a constituent substance of the spermatozoon (the sperm receptors) with the fertilizin, and that the activation expressed itself by consequent union of the fertilizin with certain egg substances (the egg receptors). The reaction was thus conceived in terms of the Ehrlich side-chain theory, and was represented diagrammatically accordingly.

That certain chemical combinations form an essential feature of the fertilization reaction cannot be open to doubt. I have not previously taken into account the consideration that the

occurence of such reactions, taking place, as they must, across the egg membrane, is dependent on physical conditions of the membrane, especially its permeability to the substances concerned. In speaking, as I did, of five blocks to the fertilization reaction, I was concerned only with the chemical reactions involved. There may be other blocks of a physical nature. Indeed these were much in evidence in the fertilization of *Asterias*, which I studied in the first part of the summer, and shall report on elsewhere. Another important consideration is that the reaction must also be dependent on environmental conditions such as temperature, ionic constitution of the medium (see Loeb, '14*b*), etc. Blocking of fertilization may also arise from such causes.

Continuing the exposition of the theory; I identified the fertilizin of *Arbacia* with the substance found in the fluid of egg suspensions which causes agglutination of sperm suspensions of the same species. This phenomenon cannot possibly be lacking in significance, for it furnishes direct evidence of a combination of egg and sperm derivatives; the phenomenon itself is not concerned in fertilization, for a single spermatozoon may fertilize an egg. Neither does the absence of such agglutination in other species affect in the least the conclusion that may be drawn from *Arbacia:* because we may have a combination of egg and sperm derivatives without any sperm agglutination. The agglutination is incidental, the combination is the essential thing.

The fertilizin theory in its essential aspects is not dependent on the identification of fertilizin and sperm agglutinating substance. I believe in their identity; but if it were proved, as Loeb has sought unsuccessfully to do, that the agglutinating substance is not essential for fertilization, the fertilizin theory would still not be attacked in its essence. The conception that initiation of development is essentially a phenomenon of activation would still stand in opposition to theories of external agents acting directly by corrosion (cytolysis), or coagulation, or what not. The egg could still be regarded as a self-contained system with no more than the usual environmental relations. It is only from this point of view that the complex phenomena of parthenogenesis and fertilization can be united in a logical whole.

The theory of the identity of fertilizin and sperm agglutinating substance rests upon a considerable body of ascertained facts (see study VI), and it gives us at once a point of attack and a working hypothesis of considerable value. I have been able to show for instance that the origin of the capacity of the egg for being fertilized can be understood on this basis; that the cessation of fertilization capacity can also be so understood; and that the physiological sterility (prevention of polyspermy) of fertilized eggs is readily explained by the neutralization of the fertilizin by a substance (anti-fertilizin) demonstrably present in the egg.

On the other hand the theory does not postulate that the fertilizin of all forms should agglutinate sperm of its own species. There may be many forms in which the union of the sperm receptors with fertilizin does not produce such physical changes of the spermatozoa as to lead to agglutination. In those cases in which agglutination does occur we have a reaction very useful in analysis; but it cannot be too strongly emphasized that the agglutination itself is to be regarded merely as an indicator of the essential reaction.

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